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Fibre-based ratiometric fluorescence imaging for contrast enhancement of spectrally similar signals in the lung

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Abstract: We present a widefield ratiometric fibred optical endomicroscopy platform capable of enhancing contrast between spectrally similar pathologically relevant fluorophores. We demonstrate the detection of fluorescently labelled bacteria in an autofluorescent *ex vivo* human lung model.

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1. Introduction

Fluorescence microscopy has demonstrated itself as a powerful tool for visualising biological pathways. Further, optical endomicroscopy (OEM) can be enabled with coherent fibre bundles (CFBs) to provide a low-cost and minimally invasive solution for real time microscopic imaging *in vivo* [1,2]. The CFBs are small and flexible enough to perform microscopy of various organs of the body, such as the lungs, for *in situ* imaging within a clinical setting. The Proteus project (www.proteus.ac.uk) aims to improve the detection and diagnosis of pulmonary infection and inflammation by developing and employing targeted fluorescent molecules (Smartprobes) for labelling specific pathologies in tissue which can be imaged using a CFB-enabled OEM system [2,3]. However, lung tissue autofluorescence from structural proteins is broad and can overlap with the spectral signatures of Smartprobes, hampering confident detection, see Fig. 1. This is particularly true for many well-established fluorophores that reside in the green region of the spectrum, which we call the green-on-green problem. We have previously demonstrated multi-colour fluorescence lifetime imaging to tackle this [3]. To more simply overcome this challenge, a novel CFB-based imaging system and accompanying image processing software that is capable of performing ratiometric imaging of tissues using two spectral bands and a single illumination wavelength was developed. We demonstrated this on an *ex vivo* human lung model instilled with Smartprobe labelled bacteria. More details can be found in [1].

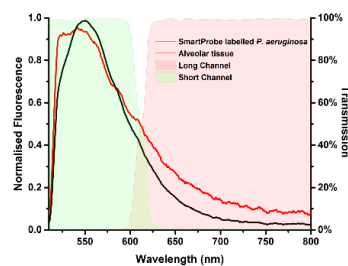


Fig. 1 Example fluorescence emission of Smartprobe labelled *P. aeruginosa* (black) and human lung (red) at excitation of 470 nm. Smartprobe labelled bacteria have greater short wavelength contributions than typical lung tissue autofluorescence. Profile of dichroic mirrors used to separate the short and long channels determines the short channel wavelength range (green fill) and the long channel wavelength range (red fill). Adapted from [1].

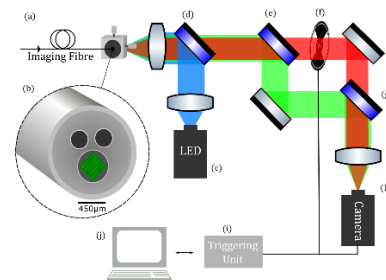


Fig. 2 Ratiometric imaging system. CFB (a) consisting of a tessellated array of 8100 cores (b). A blue LED (c) is coupled via a dichroic mirror (d) into the fibre bundle. The fluorescence emission is separated by a second dichroic mirror (e) about 605 nm. The long wavelength path is interrupted by an optical chopper (f) and recombined with another dichroic mirror (g) onto a monochromatic camera (h). A PC (j) is used to control a triggering unit (i) with outputs to the camera and the chopper. Adapted from [1].

2. Methodology

Our system, shown in Fig. 2, is enabled by a novel CFB developed by the University of Bath [4,5]. The CFB consists of 8100 cores with a 450 µm corner to corner field of view (FOV) and features two capillary channels (Fig. 2 b). The system makes use of a single colour LED illumination source (470 nm) and splits fluorescence from the tissue and Smartprobes into two optical paths, above and below a cut-off wavelength, by a 605 nm longpass dichroic

mirror. A triggered system of a monochrome CMOS camera and optical chopper allows collection of dual images of the same FOV from different parts of the spectrum. Contrast enhancement with the two bands is carried out by extracting signal per fibre core [6] and post processing to reconstruct an image, explained fully in [1]. For demonstration, a whole human lung was ventilated and a bronchoscope inserted into the airways, as described in [2]. The working channel of the bronchoscope was then used to pass our CFB into the distal alveolar regions of the lung. Baseline imaging of lung tissue was carried out, see Fig 3., before *in situ* delivery via the CFB capillary channel of Smartprobe labelled *P. aeruginosa* ($100\ \mu\text{L}$, $10^8\ \text{CFU mL}^{-1}$), similar to that described in detail elsewhere [7].

3. Results and Discussion

Imaging was continuously performed in the FOV at 10 fps in the lung region where SmartProbe labelled *P.aeruginosa* was delivered and an increase in spectral ratio suggesting the presence of bacteria could be immediately visualized, see Fig. 3. With a scale range chosen to highlight this contrast, yellow features in the regions where we delivered labelled bacteria became apparent, absent from the images of lung tissue alone. Significant spectral variation of lung tissue can also be observed with this system with alternate scaling (shown in [1]).

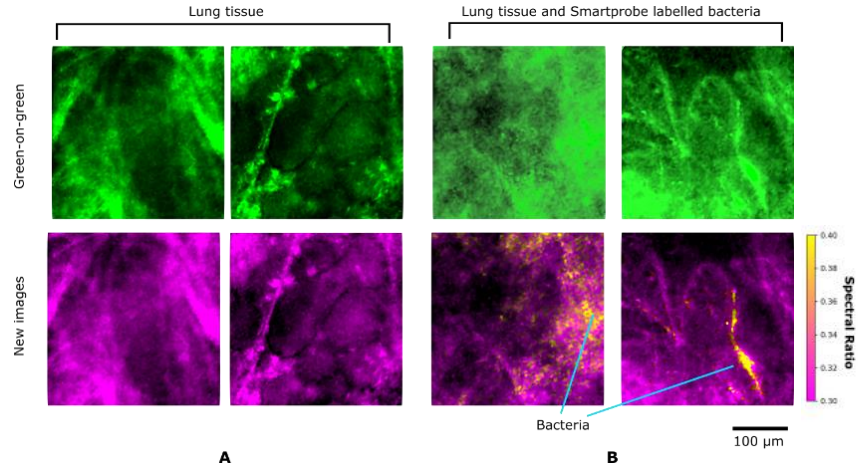


Fig. 3 Contrast enhancement of spectrally similar signals achieved with our system. **A** shows baseline lung tissue both without (top) and with (bottom) contrast enhancement. **B** shows lung tissue after addition of labelled bacteria both without (top) and with (bottom) contrast enhancement. Lung tissue appears purple. Yellow regions indicate presence of bacteria. Adapted from [1].

4. Conclusion

We have presented a CFB-enabled OEM platform with application to video rate imaging of pathologies in the human lung. We can detect targets with overlapping spectral signals using a single illumination band and this was achieved in a cost-effective manner. By coupling this simple system with a single use disposable packaged fibre we have provided an entire platform with significant clinical utility.

5. References

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